Fungus Populations in Marine Waters and Coastal Sands of the Hawaiian, Line, and Phoenix Islands[1]

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ABSTRACT: Saprophytic and facultative parasitic fungi present in the coastal waters and adjacent pelagic areas of the Hawaiian Islands, and in coastal sands of the Hawaiian, Line, and Phoenix islands, were isolated by plating methods. Isolates from all areas sampled indicate that abundant and varied fungus populations do exist in these environments. The number of fungi obtained from the inshore neritic zone was seven times that obtained from the oceanic zone. The fungus *Aureobasidium pullulans* (De Bary) Arnaud was isolated repeatedly from oceanic waters. A comparison is made between the genera and the average number of isolates per liter of water known from the Atlantic Ocean with those found in this study of the Pacific Ocean. The number of fungi isolated from sand samples of the different islands ranged from 2 to 1,600 per gram. Species diversity was evident throughout the samples. The leeward Hawaiian islands had a higher average number of isolates per gram than any other island group. In conclusion the problems of defining a marine fungus are discussed.

Oceanic areas in different parts of the world have been shown to be habitats for marine fungi (Johnson and Sparrow, 1961). Investigators, however, have usually concentrated on particular groups of fungi by use of selective isolation methods (Barghoorn and Linder, 1944; Moore and Meyers, 1959; Jones, 1962). Only one extensive analysis of marine waters for a general fungus population is known, and it was made in the northwestern subtropical Atlantic Ocean (Roth et al., 1964). References to the occurrence of fungi in the Pacific Ocean are found (1) as incidental to studies of bacteria in marine water (ZoBell, 1946); (2) in studies of specialized fungi such as lignicolous fungi (Cribb and Cribb, 1955, 1956, 1960; Kohlmeyer, 1960; Meyers and Reynolds, 1960) and those on algae (Cribb and Cribb, 1955, 1956, 1960); and (3) in studies of particular kinds of fungi, e.g., Phycomycetes in Japanese waters (Kobayashi, 1953) and pathogenic species (Van Uden and Castelo Branco, 1961). Reports of fungi from terrestrial environments of islands in the central and southern Pacific also are very limited. These include a few records of higher fungi collected in the Marshall Islands (Rogers, 1947), the Society Islands (Olive, 1957, 1958), and Raroia in the Tuamotu Archipelago (Cooke, 1961); Phycomycetes recovered by plating soils of the atolls of Bikini, Eniwetok, Rongerik, and Ronggelap (Sparrow, 1948); and Ascomycetes and Fungi Imperfecti from dung and soil samples collected by Olive in the Society Islands (Petersen, 1960). Consequently, as Cooke points out, the geographic distribution of fungi occurring on the islands of the Pacific is poorly known. Although studies have been initiated on the soils of the Hawaiian Islands (Baker, 1964) and the phyllosphere (Marsh, 1965), no study has been made of fungi occurring in marine waters and intertidal environments of these islands or elsewhere in the central Pacific. This investigation was undertaken to determine the occurrence and distribution of the saprophytic and facultative parasitic fungi which constitute the fungal populations of these habitats.

Materials and Methods

Collection

Isolations for fungi were made from 59 water samples and 50 sand samples collected

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from inshore areas of the Hawaiian Islands and adjacent pelagic environments. Seventeen sand samples were taken from the Phoenix and Line islands. The water samples were taken from four zones: the surf or spray zone; the inshore neritic zone, 2–200 m from shore; the offshore neritic zone, 300 m to 2 km from shore; and the oceanic zone, 2 km or more off shore. Of these water samples 56 were taken at the surface and 3 were taken at depths down to 600 m. The sand samples were taken from a depth of 2 inches in the supratidal, intertidal, and subtidal zones as delimited by Hedgpeth (1957). The sample sites were selected to include a variety of shore environments: leeward, windward, or areas of special interest (e.g., South Point, Hawaii, the southernmost point in the Hawaiian Islands; Waikiki Beach, probably the most frequently used beach on Oahu; and Midway Island, the northernmost inhabited island of the Hawaiian island chain.) Other samples were obtained as opportunity offered: those from Kure Island, the Phoenix Islands, the Line Islands, and the open ocean. Figure 1 gives the geographical location of the water and sand collection sites.

The surface water samples were collected in sterile 600-ml glass bottles with plastic screw caps. The closed bottles were immersed in the water, then opened and allowed to fill with water, closed underwater and brought to the surface. The depth samples were taken by sending a plastic water sampler of the van Dorn (1956) type to the designated depths. After the sampler was brought to the deck of the ship, a sterile 600-ml bottle was filled with water from the sampler.

All sand samples were collected with sterile implements and placed in sterile 100-ml jars with plastic screw caps or in sterile polyethylene bags (NASCO Whirl-Pak, Hydro Products Co., San Diego, California).

Salinity measurements were obtained by use of Quantabs SO51 (Linayer Corp., Detroit, Michigan). Temperature was determined by standard centigrade thermometer, and depth by standard oceanographic methods.

Isolation

Two principle means of isolation were employed: the pour-plate method (Salle, 1954) and the millipore filter method (Roth et al., 1964).

Standard materials and procedures were used in the pour-plate method. Amounts plated for the water samples ranged from 0.5 ml to 5.0 ml. Dilutions of 1:10 to 1:100,000 were used for sand samples. Some samples were plated upon return to the laboratory; because of the distance between most sampling sites and the laboratory, however, most of the plating was done 24 to 48 hours after collection. All samples were kept under refrigeration until plated. A control plate, uninoculated, was set up for each medium for every plating. Plates were also exposed to the air during plating operations to determine the level of air contamination in the laboratory.

Materials for the millipore filter method included sterile cellulose-ester membranes with 0.45 μ porosity (Millipore Filter Corp., Bedford, Massachusetts). The samples were run through the millipore filter apparatus using a vacuum pump. The membranes with retained fungal elements were cultured on selective media in presterilized pastic petri dishes. The amount put through the filter ranged from 100 ml to 600 ml per sample. Controls were included for testing agar sterility and air contamination.

Several kinds of media were used: sodium caseinate agar (BBL 01-549, Fred and Waksman, 1928, modified by Potter, 1957), Roth's isolation medium (Roth et al., 1964), Fell's yeast agar (Fell et al., 1960) and mycobiotic agar (DIFCO 0689-02). All media except the mycobiotic agar were made with sea water collected at the sample sites. Bacterial growth was controlled by the incorporation of 0.05% chloramphenicol (Chloromycetin, Steri-Vial No. 65, Parke, Davis and Co.). Dilutions were made with McIlvaine's buffer solution, pH 7.0 (Machlis and Torrey, 1956). The mycobiotic agar was made with 250 ml sea water and 750 ml of distilled water in order to retain the selectivity of this medium in which chloromycetin is already incorporated.

The plates were placed in paper bags, sealed with adhesive tape, and incubated at 20°–24°C. Pour-plates were incubated for three weeks and the millipore filter plates for 10 days at room temperature (20°–24°C).

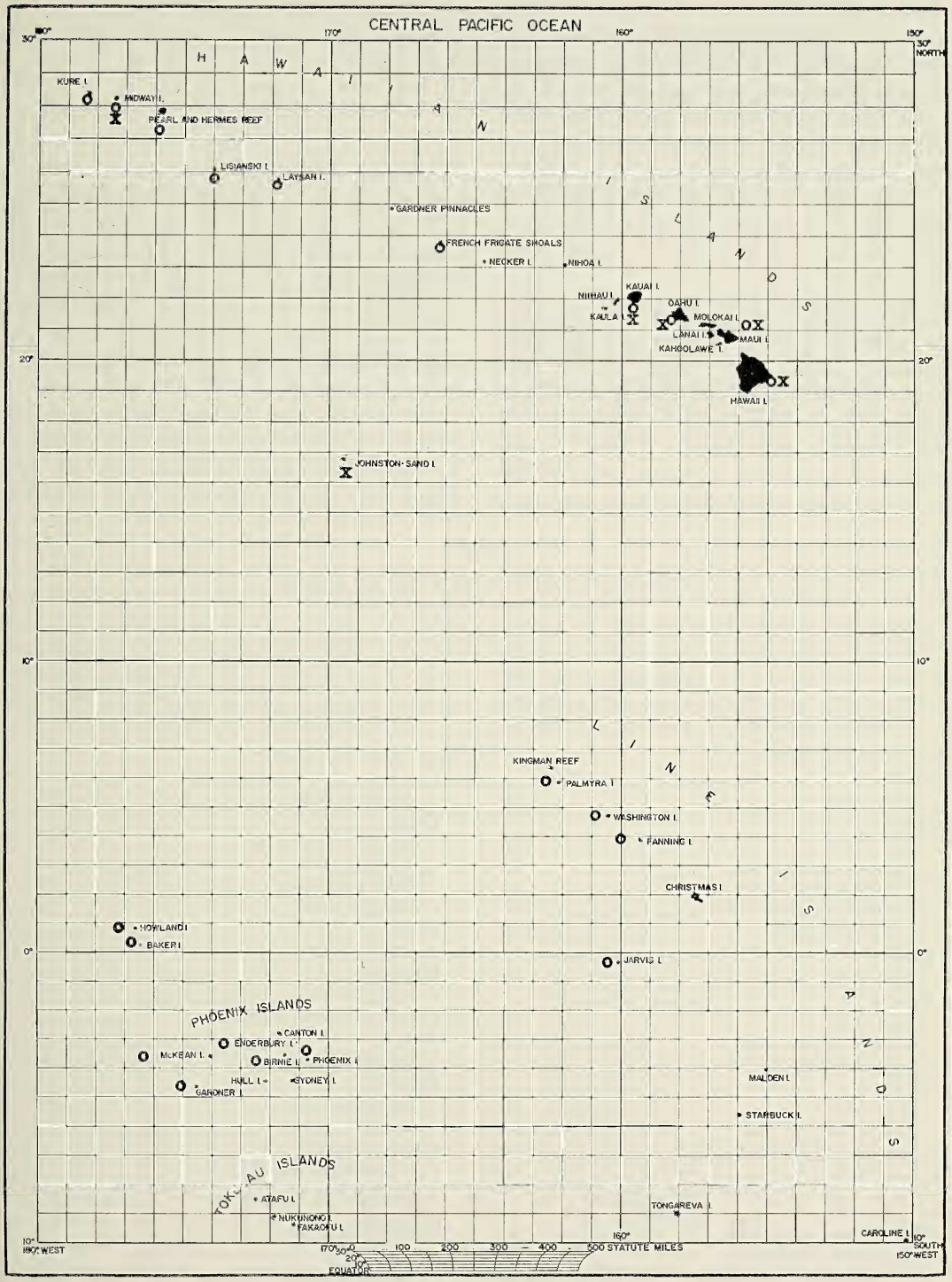

FIG. 1. General geographical location of marine water and coastal sand collection sites. *x*, Marine water collection sites; *o*, coastal sand collection sites.

Other methods of isolation, such as baiting (Johnson and Sparrow, 1961), spread-plate technique (Buck and Cleverdon, 1960) and cellulose plates made by placing a piece of sterile filter paper over an agar plate before inoculating (Baker, 1964) were attempted on some samples. The pour-plate and millipore filter methods, however, proved superior for this study.

Identification

Some fungi were identified directly from the isolation plates by a technique employing pressure sensitive tape (clear acetate tape, Scotch No. 800, Minnesota Mining and Manufacturing Co.). This method is described by Roth, Orpurt, and Ahearn (1964). Other fungi were transferred to various selective media to encourage sporulation. Those used most successfully were Czapek-Dox agar (DIFCO 0339-01) and "V-8" juice agar (Wickerham et al., 1946).

RESULTS

Populations in Water

Variations in salinity and temperature for all water sample sites was slight, ranging between 30 ‰ and 35 ‰ for salinity; between 20° and 25°C for temperature. Inasmuch as sampling was done over the period from June 1964 to May 1965 this temperature range also reflects the slight variation characteristic of a tropical climate.

All sample sites yielded fungus colonies (Table 1); 44 of the samples had counts of 50 colonies or under, whereas only 15 gave counts over 50. The standard plate count was

TABLE 1

ZONAL DISTRIBUTION OF FUNGI IN MARINE WATERS AND COASTAL SANDS

WATER SAMPLES	NUMBER OF SAMPLES	AVERAGE NUMBER ISOLATES PER ML
Main Hawaiian islands		
Kauai		
Surf zone	2	.16
Oahu		
Surf zone	9	.10
Inshore neritic zone	9	.83
Inshore bay zone	3	.28
Offshore neritic zone	3	.61
Polluted zone	3	3.18
Maui		
Surf zone	1	.17
Hawaii		
Surf zone	3	.09
Inshore neritic zone	5	.43
Leeward Hawaiian islands		
Midway		
Surf zone	1	.20
Inshore neritic zone	4	.031
Oceanic, Johnston Island	9	.066
Oceanic, Oahu	7	.029
Surf zone total	16	.12
Inshore neritic zone total	18	.34
Inshore bay zone total	3	.28
Offshore neritic zone total	3	.61
Oceanic zone total	16	.045
Polluted zone total	3	3.18
GRAND TOTAL	59	.14

SAND SAMPLES	NUMBER OF SAMPLES	AVERAGE NUMBER ISOLATES PER GM
Main Hawaiian islands		
Kauai		
Intertidal zone	5	218
Oahu		
Supratidal zone	3	185
Intertidal zone	3	6
Tidal pool zone	2	72
Subtidal zone	3	14
Maui		
Intertidal zone	1	36
Hawaii		
Intertidal zone	6	77
Leeward Hawaiian islands		
Kure		
Intertidal zone	8	41
Midway		
Supratidal zone	1	1000
Intertidal zone	6	790
Other leeward Hawaiian islands		
Intertidal zone	12	1220
Line Islands		
Intertidal zone	4	150
Phoenix Islands		
Intertidal zone	13	80
TOTAL	67	—

not significant for the dilutions used. Therefore, the number of isolates from the total number of milliliters used in plating is recorded as an average isolation return per ml of water samples. The average number of isolates from any one sample ranged from 0.06 to 3.94 isolates per ml, with the average for samples at 0.14. The number of species ranged from 1 to 17 per sample. More than 50% of the sites, however, returned only 2 to 7 different species (Steele, 1965). The predominant genera and species by percentage of occurrence are listed in Table 2.

Table 3 lists the 126 species of fungi representing 59 genera which were isolated from the water samples plated. The percentage of occurrence represents the number of water samples in which a particular fungus occurred in reference to the total number of samples analyzed. A tabulation of species isolated from the six zones sampled shows that they can be ranked in descending order for number of species per zone as follows: inshore neritic, 70; surf, 56; polluted zone, 28; oceanic, off Johnston Island, 23; oceanic, off Oahu, 20; and offshore neritic, 13. The inshore neritic zone was the richest area, having a higher average number of isolates than either the surf or oceanic zones. A very low average number of isolates was obtained from both oceanic regions. The offshore oceanic area near Johnston Island returned more isolates than the comparable zone near Oahu, but both areas had about the same number of species. *Aureobasidium pullulans* and *Rhodotorula* spp. were common to both oceanic sites. These fungi were among those predominant in all isolations from water (Table 2).

As might be expected, samples from the polluted areas off Oahu had the highest average number of fungi: 3.18 per ml. This was an area of diverse speciation. Members of the Sphaeropsidales were common, as were species of *Aspergillus, Penicillium,* and *Cephalosporium.*

Populations in Sand

From 67 sand samples plated from four different zones, 134 species of fungi representing 71 genera were recovered (Table 3). The frequency of predominant isolates by species is given as percentage of occurrence (Table 2). The average number of isolates per gm, obtained by the standard dilution plate counting

TABLE 2

PREDOMINANT GENERA AND SPECIES IN WATER AND SAMPLES

WATER	PERCENTAGE* OF OCCURRENCE	SAND	PERCENTAGE OF OCCURRENCE
Yeasts	45.8	*Aspergillus wentii*	50.7
Rhodotorula spp.	27.1	*Fusarium* spp.	44.7
Fusarium spp.	22.0	*Phialophora* spp.	25.3
Cephalosporium curtipes	22.0	*Penicillium* spp.	22.3
Cladosporium cladosporioides	16.9	*Aspergillus niger*	20.8
C. epiphyllum	16.9	Yeasts	20.8
Helminthosporium anomalum	16.9	*Megaster* sp.	17.6
Trichoderma lignorum	15.2	*Masoniella grisea*	16.4
Aspergillus niger	13.5	*Aspergillus* spp.	14.9
A. wentii	13.5	*A. terreus*	13.4
Aureobasidium pullulans	13.5	*A. ustus*	11.9
Phoma spp.	11.8	*Trichoderma lignorum*	11.9
Aspergillus spp.	11.8	*Cladosporium cladosporioides*	11.9
Black yeasts	11.8	*C. epiphyllum*	11.9
Pestalotia spp.	10.1	*Cephalosporium roseo-griseum*	11.9
Cladosporium herbarum	10.1	*C.* spp.	11.9
Aspergillus versicolor	10.1	*C. acremonium*	10.4
Penicillium spp.	10.1	*C. curtipes*	10.4
		Penicillium lilacinum	10.4

* The percentage of occurrence represents the number of water or sand samples in which a particular fungus occurred in reference to the total of 59 water samples or 67 sand samples analyzed (Orpurt, 1964).

TABLE 3

GENERA AND SPECIES ISOLATED FROM MARINE WATERS AND COASTAL SANDS

NAME	PERCENTAGE OCCURRENCE IN WATER SAMPLES	PERCENTAGE OCCURRENCE IN SAND SAMPLES
PHYCOMYCETES		
Mucorales		
Cunninghamella echinulata Thaxter		1.4
C. sp.		1.4
Mucor globosus Fischer		1.4
Rhizopus nigricans Ehrenberg	1.6	2.9
Syncephalastrum racemosum (Cohn) Schroeter	1.6	1.4
ASCOMYCETES		
Chaetomium olivaceum Cooke and Ellis		1.4
C. sp.	1.6	
Melanomma sp.		1.4
Melanospora lagenaria (Pers.) Fuckel		1.4
M. sp.		1.4
Microascus intermedius Emmons and Dodge		1.4
M. trigonosporus Emmons and Dodge		1.4
Neurospora sp.	5.0	2.9
Sporormia sp.		2.9
BASIDIOMYCETES		
Sp. indet.	1.6	
DEUTEROMYCETES		
Sphaeropsidales		
Amerosporium sp.		1.4
Aposphaeria sp.	5.0*	
Coniothyrium fuckelii Sacc.	1.6	
Cytosporina sp.	3.3	
Diplodia sp.		1.4
Diplodina sp.	1.6	
Macrophoma sp.	1.6	
Peyronellaea sp.	3.3	
Phoma hibernica Grimes	3.3	2.9
Phoma spp.	11.8	7.1†
Phomopsis sp.	1.6	
Phyllosticta sp.	1.6	
Piggotia sp.	1.6	
Pyrenochaeta sp.	1.6	1.4†
Sphaeronaema spinella Kalchb.	1.6	
Sporonema sp.		1.4
Melanconiales		
Pestalotia sp.	10.1	5.9†
Phylactaena sp.	1.6	
Moniliales		
Sporobolomycetaceae		
Sporobolomyces sp.		2.9‡
Moniliaceae		
Acremonium sp.		1.4‡
Acrostalagmus cinnabarinus Corda	1.6	1.4
Aleurisma carnis (Brooks and Hansford) Bisby		1.4†
Allescheriella crocea (Mont.) Hughes	1.6	
Aspergillus amstelodami (Mangin) Thom		1.4‡

TABLE 3 (*continued*)

NAME	PERCENTAGE OCCURRENCE IN WATER SAMPLES	PERCENTAGE OCCURRENCE IN SAND SAMPLES
A. caespitosus Raper and Thom		1.4
A. candidus Link		4.4‡
A. carneus (van Tiegh) Bloch.		4.4‡
A. clavatus Desm.		2.9‡
A. effusus Tiraboschi		1.4‡
A. flavipes (Bainier and Sartory) Thom and Church	3.3	2.9
A. flavus Link	1.6*	1.4
A. fumigatus Fres.	1.6	4.4‡
A. granulosus Raper and Thom		7.1
A. itaconicus Kinoshita	1.6*	
A. janus Raper and Thom	1.6	4.4
A. luchuensis Inui	1.6	2.9
A. micro-virido-citrinus Cost. and Lucet	1.6	
A. nidulans (Eidam) Wint.		1.4
A. niger van Tiegh.	13.5*	20.8‡
A. niveus Bloch.		1.4
A. ochraceus Wilhelm	3.3	
A. oryzae (Ahlburg) Cohn	1.6	2.9
A. panamensis Raper and Thom		2.9
A. proliferans G. Smith	1.6*	
A. restrictus G. Smith		1.4
A. ruber (Spieckermann and Bremer) Thom and Church		1.4
A. sulphureus (Fres.) Thom and Church	3.3	1.4
A. sydowi (Bain. and Sart.) Thom and Church	3.3*	7.1‡
A. tamarii Kita	1.6*	2.9‡
A. terreus Thom	3.3	13.4‡
A. unguis (Emile-Weil and Gaudin) Thom and Raper		1.4‡
A. ustus (Bainier) Thom and Church	1.6	11.9‡
A. versicolor (Vuill.) Tiraboschi	10.1*	5.9‡
A. wentii Wehmer	13.5*	50.7†‡
A. spp.	11.8*	14.9
Botryophialophora marina Linder		2.9
Cephalosporium acremonium Corda	3.3*	10.4†‡
C. asperum Marchal		1.4
C. coremioides Raillo		1.4
C. curtipes Sacc.	22.0*	10.4
C. humicola Oudemans	3.3*	1.4
C. roseo-griseum Saksena	6.7*	11.9‡
C. sp.	1.6	11.9
Fusidium viride Grove	1.6	1.4
Gliocladium fimbriatum Gilman and Abbott		1.4
Malbranchea sp.		1.4
Moeszia sp.	1.6	
Monilia brunnea Gilman and Abbott		1.4
Monocillium sp.	1.6	5.9‡
Paecilomyces fusisporus Saksena	1.6*	
P. varioti Bainier	1.6	2.9
Penicillium albidum Sopp		1.4
P. brevi-campactum Dierckx	3.3	
P. canescens Sopp	3.3	
P. caseicolum Bainier	1.6	1.4
P. charlesii Smith	1.6	1.4‡

TABLE 3 (*continued*)

NAME	PERCENTAGE OCCURRENCE IN WATER SAMPLES	PERCENTAGE OCCURRENCE IN SAND SAMPLES
P. chermesinum Biourge		1.4
P. citrinum Thom	1.6	8.9‡
P. commune Thom	1.6	
P. corylophilum Dierckx		1.4‡
P. cyaneo-fulvum Biourge	1.6	
P. cyaneum (Bainier and Sartory) Biourge	1.6	
P. janthinellum Biourge	3.3	
P. kapuscinskii Zaleski		1.4‡
P. lanosum Westling	6.7	1.4
P. lanoso-coeruleum Thom	5.0	
P. lilacinum Thom	6.7*	10.4
P. miczynskii Zaleski		1.4
P. nigricans (Bainier) Thom	8.4	8.9‡
P. notatum Westling	5.0	
P. oxalicum Currie and Thom	1.6	1.4
P. piscarium Westling		1.4
P. purpurrescens Sopp	1.6	
P. raciborskii Zaleski	1.6	
P. rotundum Raper and Fennell		1.4‡
P. simplicissimum (Oud.) Thom	1.6*	
P. steckii Zaleski	1.6	4.4‡
P. velutinum van Beyma		2.9
P. spp.	10.1*	22.3†‡
Rhinotrichum sp.		1.4†‡
Scopulariopsis brevicaulis Bainier		4.4‡
S. brumptii Salvanet-Duval		1.4‡
S. carbonaria Morton and G. Smith	1.6	
S. croci van Beyma		1.4‡
S. fimicola (Cost. and Mat.) Vuill.	1.6	2.9
S. sp.	1.6	7.1
Sepedonium sp.	1.6	2.9
Spicaria simplicissima Oudemans	1.6*	
Sporotrichum epigaeum Brunard		2.9
Trichoderma album Preuss	3.3	
T. glaucum Abbott	1.6	2.9
T. koningi Oudemans	5.0*	1.4
T. lignorum (Tode) Harz	15.2*	11.9
Trinacrium sp.		1.4
Tritirachium album Limber		1.4
T. purpureum (Saito) Beyma	3.3*	
Varicosporium sp.		1.4
Verticillium candelabrum Bonorden	1.6	
V. terrestre (Link) Lindau	3.3	1.4
Dematiaceae		
Acrostaphylus sp.		1.4
Acrotheca sp.		1.4
Alternaria fasciculata Cooke and Ellis	1.6	1.4
A. geophila Daszewska	1.6	
A. humicola Oudemans	3.3	
A. tenuis Nees	3.3	
Aureobasidium mansonii (Cast.) Cooke		1.4‡
A. pullulans (De Bary) Arnaud	13.5*	8.9†‡
A. sp.	1.6	4.4‡

TABLE 3 (*continued*)

NAME	PERCENTAGE OCCURRENCE IN WATER SAMPLES	PERCENTAGE OCCURRENCE IN SAND SAMPLES
Bispora sp.		1.4
Catenularia sp.	1.6	
Chalaropsis sp.	1.6	
Chloridium sp.	1.6	
Cladosporium cladosporioides (Fres.) de Vries	16.9*	11.9†‡
C. epiphyllum Persoon	16.9*	11.9
C. herbarum (Persoon) Link	10.1	8.9†‡
C. lignicolum Corda	3.3	
C. spp.		10.4
Cordana sp.		2.9†
Curvularia geniculata (Tracy and Earle) Boedijn	6.7	1.4‡
C. interseminata (Berkeley and Ravenel) Gilman	3.3	
C. pallescens Boedijn	3.3	4.4
C. subulata (Nees) Boedijn	3.3*	
C. tetramera (McKinney) Boedijn	3.3	
Dendryphion sp.	1.6	
Gliobotrys alboviridis von Hohnel		1.4
Gliomastix convoluta (Harz) Mason		8.9
Gonytrichum macrocladum (Sacc.) Hughes	1.6	
G. sp.		1.4
Hansfordia togoensis Hughes	1.6	
H. sp.	1.6	
Helminthosporium anomalum Gilman and Abbott	16.9	1.4
H. sativum Pammel, King and Bakke	1.6	
Heterosporium sp.		1.4
Hormodendrum cladosporioides (Fresenius) Sacc.	1.6	
Humicola grisea Tragen	1.6	
H. lanuginosa (Griff and Maubl.) Bunce		1.4
H. nigrescens Omvik		1.4
H. sp.		2.9
Macrosporium cladosporioides Desm.	1.6	
M. sacrinaeforme Cavara	1.6	
Masoniella grisea (Smith) Smith	5.0	16.4‡
Megaster sp.		17.6†‡
Menispora apicalis Berk. and Curt.	1.6	
Nigrospora sphaerica (Sacc.) Mason	3.3	8.9†
Oidiodendron citrinum		1.4
O. griseum Robak.		1.4
Passalora sp.		1.4
Periconia byssoides Persoon		1.4
P. hispidula (Pers. ex Pers.) Mason and M. B. Ellis		1.4
Phialophora sp.	8.4	25.3†‡
Scolecotrichum sp.		1.4†
Stachybotrys atra Corda		1.4
S. lobulata Berkeley		4.4
Stemphylium botryosom Wallrath	1.6	
S. macrosporoideum (Berkeley and Broome) Sacc.	3.3	
Torula allii (Harz) Sacc.	1.6	
T. lucifuga Oudemans		4.4‡
T. sp.		1.4‡
Trichocladium sp.		1.4
Zygosporium masonii Hughes		4.4
Stilbaceae		

TABLE 3 (*continued*)

NAME	PERCENTAGE OCCURRENCE IN WATER SAMPLES	PERCENTAGE OCCURRENCE IN SAND SAMPLES
Didymostilbe sp.	1.6	
Graphium sp.		1.4
Harpographium sp.	1.6	
Synnematium jonesii Speare	3.3	1.4
Tuberculariaceae		
Cylindrocarpon didymum (Hartung) Wollenweber		2.9†
C. radicicola Wollenweber		1.4
Epicoccum purpurascens Ehrenb.	1.6	
E. sp.		2.9
Fusarium merismoides Corda	1.6	
F. spp.	22.0*	44.7†‡
Hymenella sp.		4.4†
Myrothecium roridum Tode	3.3	
M. verrucaria (Alb. and Schw.) Ditmar ex Fr.		1.4
M. sp.	1.6	1.4
Yeasts		
Rhodotorula spp.	27.1*	1.4
Orange yeasts	1.6	8.9†‡
Pink yeasts		2.9†
Black and orange yeasts		1.4
Black yeasts	11.8*	8.9†‡
Yeasts	45.8*	20.8†‡
Mycelia sterilia (Dematiaceae)	28.8*	37.3†‡
Mycelia sterilia (Moniliaceae)	16.9*	40.1†‡

* Isolated from oceanic zone.
† Isolated from the Line Islands.
‡ Isolated from the Phoenix Islands.

technique, is given in Table 1. A total of 37 samples had fewer than 100 isolates per sample; the remaining 30 samples had more than 100 isolates per sample. The sand of the leeward Hawaiian islands had the highest number of isolates, over 500 per sample, but the number of different species was lower than in comparable samples from the Phoenix Islands.

The sands returned from 1 to 35 species per sample. The majority yielded from 3 to 9 species each. The intertidal sands of the main Hawaiian islands returned the highest number of species, a total of 68. Black Sand Beach, Hawaii, and Kaena Point, Oahu, each yielded totals of 16 species. The supratidal zone of Kuhio Beach, Oahu had the highest number of species for sites in that zone: 35 species among 16 genera. The zone total was 53 species. The subtidal zone returned the lowest number of species, only 22.

In Table 2, the 18 fungi occurring most frequently in water and sand are listed. Among these, 9 are common to both areas although of different rank for percentage of occurrence; 7 occur in water and not sand, and 8 occur in sand, not in water. Of those common to both water and sand, yeasts, aspergilli, and penicillia were common to all sand samples. Neither *Rhodotorula* spp. which is penultimate in rank for water, nor *Aureobasidium pullulans,* also frequent in water samples, was predominant for sand samples.

The control plates poured when both water and sand samples were plated showed no growth. Only one colony was observed on a plate exposed to determine the level of air contamination in the laboratory.

DISCUSSION

Isolates obtained from water samples indicate that abundant and varied fungus popula-

tions do exist in this environment, but that frequencies vary with zones. The oceanic zone had fewer isolates than did the other zones studied in the pelagic region. This result was expected, inasmuch as oceanic regions are known to have lower populations of marine organisms than do regions closer to land. Of the 59 water samples, 50 were taken from areas that are strongly influenced by the presence of oceanic islands, whereas the other 9 were from areas well away from any shore. Areas near islands are known to support abundant and varied marine life. Ships may also be a source of water pollution, and therefore modified populations might be expected in shipping lanes.

Differences can also be observed between the two oceanic locations studied, as reflected in differences in kind of fungi present but not in numbers. The oceanic area off Johnston Island contained more yeasts than did surface samples obtained near the island of Oahu. The latter had more fungi which would be classified as terrestrial, such as aspergilli and penicillia. The high yeast population observed from the oceanic areas is in agreement with the findings of both ZoBell (1946) and of Fell and Van Uden (1963). Members of the genus *Rhodotorula* were isolated consistently from all water samples, including the depth samples. Roth et al. (1962) have noted the common occurrence of these yeasts in oceanic localities, an observation now confirmed by these studies for the Pacific Ocean.

When the three zones—surf, inshore neritic, and offshore neritic—are compared, a correlation is found between numbers of isolates per ml and location of zone. This is not unexpected. This correlation is particularly clear in the data for these zones in Oahu. The surf zone, which is an unstable area with constant wave action, returned 0.10 isolates per ml compared with 0.33 for offshore neritic and 0.83 for inshore neritic zones. Species of *Curvularia, Alternaria,* and *Helminthosporium* were isolated repeatedly from the inshore neritic zone. This zone, in the area of Oahu, is richer in number of species than is the same zone on Hawaii. This might be due to pollution, as Oahu has a greater population and has a major port for shipping. Members of the order Sphaeropsidales, of the Fungi Imperfecti, were frequently found in the Oahu samples. These fungi are parasitic on plants. The polluted area contained much floating debris which could serve as a source of these fungi.

Some water samples were taken from tidal pools in the intertidal zone. In every case there was a high number of isolates. Intertidal pool populations may be affected by higher temperatures, higher organic content, salinity levels, or wave action. One area, however, that of a large bay on the windward side of Oahu, did not return a high yield of isolates. This area has a high fresh-water run-off which reduces the salinity at the surface as much as 5 ‰ during the rainy season. Even though the area had a lower population than expected, it did have great diversity, explained perhaps by the run-off factor. The high number of aspergilli and penicillia isolated was to be expected because of their proclivity for sporulation, cosmopolitan habitat, and their great adaptability. A good percentage of these might be run-off and/or air contaminants introduced into the water.

The fact that more fungi were isolated from the sand than from the water supports the well-known observation that microbial populations are higher in relation to fixed surfaces. Examination of Table 3 also shows that the general population of sand is quite different than that of water.

The high number of isolates from the sand samples taken from the leeward Hawaiian islands and the few species among them is in direct contrast with the low number of isolates and high number of species in the samples from the Phoenix Islands and the Line Islands. There could be many reasons for this. The elapsed time before plating the leeward samples was greater than for those of the Phoenix and Line islands. Variations in bird population, temperature, and humidity, and shore stability among the leeward Hawaiian islands, may be critical factors controlling fungus populations. If the bird population serves as a control, a survey for keratinophilic and coprophilous species might be rewarding. Such a survey should also extend to other islands with large bird populations. Another factor influencing fungus populations, as reflected in the high number

of species returned from the Phoenix and Line islands, is the fact that Gardner Island, Washington Island, and Palmyra Island are, or have been, inhabited.

The supratidal zone gave the highest average number of isolates per gm. This zone is a very stable area, a mixture of soil and sand. When the average number of isolates is compared for the supratidal, intertidal, and subtidal zones on Oahu, the intertidal displays the lowest average number of isolates, which may be explained by the influence of constant washing by the waves. The tidal pool area of the sand, like the tidal pool area of the water, is characterized by the presence of more fungi than are found in the surrounding areas of each zone.

Two of the sand areas sampled are unique among sands for their color and composition, namely a green sand beach and a black sand beach. The black sand beach had 259 isolates distributed through 12 genera and 200 species, while the green sand beach had 28 isolates, in 10 genera and 12 species. Although 4 genera were common to both, only 1 species, *Aspergillus terreus,* occurred at both sites. Both beaches are on the island of Hawaii. One reason for this difference may be that the black sand beach is continually exposed to contamination from people and their litter, while the green sand beach is in a very remote location. Another reason could be the difference in the chemical composition of the sands. Black sand is formed from basalt lava rock and cinders, and green sand is formed by the release of olivine crystals which are in the basalt lava rock.

Most of the Phycomycetes and the Ascomycetes were isolated from sand. These fungi are known to adhere to substrates. Only one species predominates over the others, *Aspergillus wentii,* as shown in Table 2. When plating four samples on mycobiotic agar from beaches that are local tourist attractions on Oahu, one potential pathogen was found: *Microascus intermedius,* which has been isolated from a number of soils by mouse passage. Several species of this genus are known to be etiologic agents of dermatophytoses and onychomycosis in man (Barron et al., 1961).

It is interesting to compare the results obtained by Roth et al. (1964) in the Atlantic with the results obtained from this study in the Pacific. Roth took 227 water samples and identified 41 genera among his isolates. This study encompassed 59 water samples and resulted in the identification of 59 genera. Of these, 29 genera were common to both lists. The Atlantic list included 11 genera not reported in this study; this study includes 29 genera not reported by Roth et al. (1964).

Table 4 shows the difference in average number of isolates per liter between the samples taken in the Atlantic Ocean and those taken in the Pacific Ocean.

Furthermore, it is a striking fact that no Pacific sample, either water or sand, was without fungi, whereas Roth et al. (1964) recovered fungi from only 80% of their 227 samples. Because they did not include sand samples in their study, comparisons can be made only between water samples. The maximum number of

TABLE 4

COMPARISON OF NUMBERS OF ISOLATES FROM THE ATLANTIC OCEAN AND THE PACIFIC OCEAN

ATLANTIC OCEAN (ROTH ET AL. 1964)			PACIFIC OCEAN (1965)		
DEPTH	NUMBER OF SAMPLES	AVERAGE NUMBER ISOLATES PER LITER	DEPTH	NUMBER OF SAMPLES	AVERAGE NUMBER ISOLATES PER LITER
500 to	5	11.1	600 m	1	13.0
1000 m	17	12.1	300 m	1	6.0
	9	4.9	300 m	1	10.0
	2	1.0			
Surface	12	15.5	Surface	1	18.0
to	79	17.5		1	55.0
500 m	43	15.1		1	30.0
	16	3.0		1	73.0

species per offshore sample in the Atlantic was 6. In the Pacific the number ranged from 1 to 11. Although the maximum number of species per Pacific sample exceeds that reported for the Atlantic, the total number of species found in each area is approximately the same: 133 species in the Atlantic compared with 127 in the Pacific. The diversity of genera is notably higher in the Pacific samples. Samples from both areas yielded fungi that were not identified.

There are both differences and similarities between the kinds of fungi obtained from the two regions. A total of 30 species were common to Atlantic and Pacific waters. Neither Ascomycetes nor Basidiomycetes were reported for the Atlantic. From the Pacific water 2 Ascomycetes and 1 Basidiomycete were recovered. Roth et al noted the low incidence of Phycomycetes, reporting only 6. Five species were identified in this Pacific study, but only 2 were from water samples. Species of Sphaeropsidales occurred in similar numbers in both oceans: 7 in the Atlantic and 5 in the Pacific, but only 2 species were common to both. In comparing the species of fungi which are predominant in each area, some species are found on both lists. Roth et al. distinguished 8 fungi each from the eulittoral and oceanic water samples as their dominant species. Of these, *Aureobasidium pullulans* was most common in the oceanic zone and it did not occur in their eulittoral list. In the Pacific, this fungus was common in water but ranked seventh among 18 species. *Cladosporium* species (*sic*) were the most common fungi in the Atlantic eulittoral samples, and occupied second place in the oceanic list. Two species, *Cladosporium cladosporioides* and *C. epiphyllum,* ranked high among those frequent in both water and sand samples of the Pacific. *Trichoderma lignorum,* the last species on the Atlantic list of eulittoral dominants, is absent from the corresponding oceanic list. In the Pacific it occurred in both water and sand samples, although much more frequently in water than in sand. In summary, the differences suggest that the Pacific fungus population is different from that of the Atlantic.

Having established the fact that fungi can be isolated from Pacific as well as Atlantic water and shores, there still remains the problem of how one determines whether or not a certain isolate is a marine fungus. There is no single diagnostic test. The criteria which have been suggested are summarized by Roth et al. (1964) and may be stated as: (1) the isolate must grow and reproduce exclusively or predominantly in the sea or on intertidal substrata, or (2) the isolate must grow and reproduce at an optimal level in the normal salinity of the oceans. None of these criteria can be supported without qualification. There is still no means of concrete demonstration of growth and reproduction of a fungus in vivo in marine environments except for those fungi growing on natural (and introduced) substrata. If growth and reproduction at normal salinity levels is accepted as a criterion, this allows for the inclusion of fungi found in salt lakes (Anastasiou, 1963). Moreover, Gray (1963) has shown that many fungi of terrestrial origin are capable of better growth in sea water media than in fresh water media. If growth is limited to natural substrata, then the possibility of free-living marine fungi is excluded.

As more investigations of marine habitats are undertaken, the number of genera and species isolated from them will undoubtedly increase. Many of the genera found in the Pacific are known from other marine locations, e.g., species of *Aureobasidium, Macrophoma, Phoma, Diplodina, Diplodia, Epicoccum, Fusidium, Cladosporium, Alternaria,* and *Macrosporium* (Johnson and Sparrow, 1961). Repeated isolation, however, is not confirmatory evidence, but is only suggestive. Roth et al. (1964) contend that, until further distributional and physiological data are obtained, fungi so isolated should be regarded as of incidental occurrence in the sea. Nor is the rare isolation of a species from only marine habitats reliable evidence, as this may reflect only the randomness of the sampling and the samplers. Curiously, *Dendryphyiella arenaria* Nicot., which is dominant in Atlantic eulittoral samples and known only from marine sources, was not among the Pacific isolates. Conversely, *Botryophialophora marina,* also known only from marine sources, was found in the Pacific but not in the Atlantic.

In conclusion, a working definition for a marine fungus is proposed: A marine fungus is one which is capable of producing successive generations by sexual and/or asexual means in

natural oceanic waters or on intertidal substrata. Until experimental means of proof are devised, the data presented here will serve as contributory evidence for the distribution of fungi isolated from marine habitats.

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